



TITLE:

Poly(ADP-ribosyl)action and ischemia in brain/ Cloning and characterization of LUN, a novel RING finger protein/
Introduction of neuronal apoptosis and generation of reactive oxygen species by NAC amyloid (BIOORGANIC CHEMISTRY - Molecular Clinical Chemistry)

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Bioorganic Chemistry -Molecular Clinical Chemistry-



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Scope of Research

This laboratory was founded in 1994 with the aim of linking (bio)chemical research and clinical medicine. Thus, the scope of our research encompasses the structure, function and regulation of various biomolecules, the pathophysiological significance of bioreactions in relation to human diseases, and the application of molecular techniques to clinical diagnosis and therapy. Our current interest is focused on poly(ADP-ribosyl)ation, nuclear localization of proteins in association with apoptosis, and the molecular etiology of cancer and neurodegenerative disorders including Alzheimer's disease.

Research Activities (Year 2001)

Presentations

A possible role of poly(ADP-ribose) synthetase in neuronal degeneration.

Ueda K. and Tanaka S. 21st Annual Meeting of the Australian Neuroscience Society in conjunction with the Asian Pacific Society for Neurochemistry, Brisbane, 28-31 January, 2001.

The effect of organic solvents on poly(ADP-ribose) synthetase activity: Implications for risk assessment.

Banasik M., Stedeford T. and Ueda K. SEPATEC/Asia-Pacific Symposium 2001. Kanazawa, 1-2 November, 2001.

Grants

Ueda K. Special Coordination Funds for Promoting Science and Technology from the ministry of Education, Culture, Sports, Science and Technology. 1 April 1998 - 31 March 2003.

Ueda K. A role of poly(ADP-ribose) synthetase in recovery from DNA damage. Japan Foundation for Applied Enzymology. 1 April 2001 - 31 March 2002.

Topics

Poly(ADP-ribosyl)ation and ischemia in brain

Nitric oxide from neuronal cells plays detrimental roles in glutamate neurotoxicity and focal brain ischemia. Nitric oxide directly damages DNA, and breaks in the DNA strands activate poly(ADP-ribosyl)ation of nuclear proteins. The excessive activation of poly(ADP-ribose) synthetase (PARS) is thought to cause depletion of ATP and the energy failure leading to cell death. To clarify the involvement of poly(ADP-ribosyl)ation in ischemic insult, we examined poly(ADP-ribosyl)ation by immunohistochemical methods and tested the protective effect of 3-aminobenzamide, which is a PARS inhibitor, on focal brain ischemia using a rat model of permanent middle cerebral artery occlusion. Poly(ADP-ribosyl)ation was widely and markedly detected 2 hours after the ischemic insult in the cerebral cortex and striatum where infarction developed 24 hours later. The enhanced immunoreactivity of poly(ADP-ribose) gradually decreased, and 16 hours later, no immunoreactivity was detected. Intraventricular administration of 3-aminobenzamide 30 min before the ischemic insult decreased infarction volume in a dose-dependent manner along with the immunohistochemical reduction of poly(ADP-ribosyl)ation. Pretreatment with 7-nitroindazole, a selective neuronal nitric oxide synthetase inhibitor, partially reduced poly(ADP-ribosyl)ation. These data suggest the involvement of poly(ADP-ribosyl)ation in the development of cerebral infarction.

Ref. Tokime, T., *et al.* J. Cerebral Blood Flow Metab. 18(9), 911-997 (1998)

Cloning and characterization of LUN, a novel RING finger protein

We isolated cDNAs encoding a novel RING finger protein (LUN), the mRNAs of which were expressed at high levels in the lung. *In situ* hybridization revealed that LUN mRNAs were expressed in the alveolar epithelium of the lung. The *LUN* gene locus was assigned to chromosome 9p21, which contains candidate tumor suppressor genes associated with loss of heterozygosity in more than 86% of small cell lung cancers. We clarified that LUN is localized to the nucleus and has a Zn^{2+} -dependent DNA binding activity. The amino acid 51 - 374 region of LUN is responsible for the DNA binding. Furthermore, we identified a novel palindromic binding consensus (5'-TCCCAGCACTTTGGGA-3') for the LUN binding. Interestingly, this LUN binding palindromic sequence is found in the upstream transcriptional regulatory region of

the E-cadherin gene and two intervening regions of the *tal* gene. Our results suggested that LUN might be an important *trans*-acting transcriptional regulator for lung cancer-associated genes including E-cadherin and *tal* genes.

Ref. Chu, D., *et al.* J. Biol. Chem. 276 (17), 14004-14013 (2001).

Induction of neuronal apoptosis and generation of reactive oxygen species by NAC amyloid

Amyloid deposition in senile plaque cores is one of the histopathological changes characteristic of Alzheimer's disease (AD). The AD amyloid consists of A β protein and many minor substances, including the non-A β component (NAC) of AD amyloid. NAC is a very hydrophobic peptide consisting of at least 35 amino acids derived from a precursor protein, NACP (α -synuclein). While NAC has been demonstrated to bind to A β and stimulate its aggregation *in vitro*, NAC displays a β -sheet structure and is amyloidogenic by itself. The exposure of cortical neurons to NAC fibrils induced apoptosis by generation of reactive oxygen species (ROS) in mitochondria. It also increased the nuclear translocation of NF- κ B, and enhanced its DNA-binding activity. NF- κ B is known to be activated by oxidative stress. We propose a working hypothesis that NAC interacts with A β protein to form amyloid fibrils and then the fibrils cause neuronal cell injury via ROS generation in mitochondria in AD brain.

Ref. Tanaka, S., *et al.* α -Synuclein/NACP and neurodegeneration. In "Neuroscientific Basis of Dementia" (C. Tanaka, *et al.* eds.), Birkhäuser Verlag, Basel, 137-141 (2001).

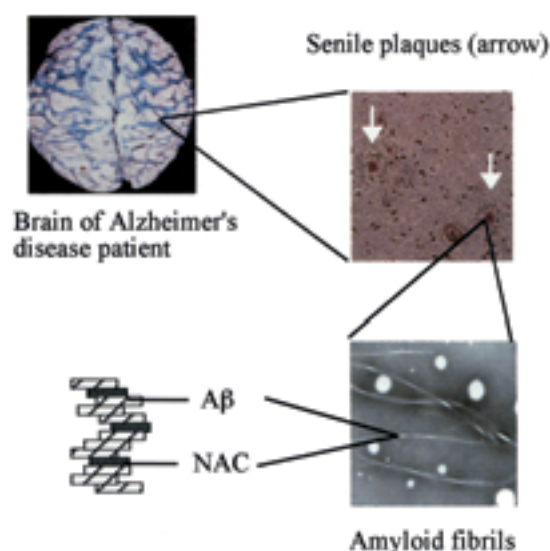


Fig. 1. NAC / A β amyloid in Alzheimer's disease brain